Vol. 95, No. 2, 1980
July 31, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 513-519

FLUOROPYRUVATE: A POTENT INHIBITOR OF THE BACTERIAL AND THE MAMMALIAN PYRUVATE DEHYDROGENASE COMPLEX

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Received May 28,1980

SUMMARY

Fluoropyruvate appears to be a very strong inhibitor, competitive to pyruvate, of the pyruvate dehydrogenase complexes both from Escherichia coli (K_i = 1.4 µM) and from bovine kidney (K_i = 3.6 µM). While the saturation kinetics of pyruvate for the bacterial enzyme complex have a sigmoidal characteristic (K_m = 0.3 mM), the binding of the inhibitor exhibited a normal hyperbolic function. In comparison, the mammalian enzyme complex shows normal Michaelis-Menten kinetics for pyruvate (K_m = 0.06 mM).

Fluoropyruvate is able to replace pyruvate as a substrate in the enzymatic catalysis of pyruvate carboxylase (1), lactate dehydrogenase (2), and the pyruvate dehydrogenase complex (3). In this latter case, an unusual partial reaction involving the first enzyme component, but not the overall reaction of the complex, can be observed with this substrate analog. The strong metabolic inhibitory effect found for fluoroacetate (4, 5) has as yet not been observed for fluoropyruvate leading to the assumption that this compound is less toxic than fluoroacetate (6).

In the course of kinetic studies with the pyruvate dehydrogenase complexes both from bovine kidney and from E. coli, a very strong inhibitory effect of fluoropyruvate, competitive to pyruvate, was observed for the overall enzymatic reaction. This enzyme may therefore be considered to be the main target of the metabolic block seen when the cell is exposed to fluoropyruvate.

0006-291X/80/140513-07$01.00/0

MATERIALS AND METHODS

The substrates and cofactors for the enzyme tests were purchased from Boehringer, Mannheim, GFR, sodium ß-fluoropyruvate was from Serva, Heidelberg, GFR.

Pyruvate dehydrogenase complex from E. coli was purified according to a new rapid method (7). Purified enzyme complex from bovine kidney was a gift of Dr. G.-B. Kresze, Munich. The overall reaction rate of the enzyme complex was measured by monitoring the reduction of NAD at 339 nm and 37° C, according to the method of Schwartz et al. (8). All enzyme tests were measured in the presence of 0.1 M potassium phosphate buffer pH 7.6.

RESULTS

Pyruvate saturation kinetics; comparison of bacterial and mammalian enzyme complexes. The saturation of the pyruvate dehydrogenase complex from E. coli with pyruvate proceeds in a positive cooperative manner (9). This positive cooperative saturation is the case especially at lower concentration levels, however at and particularly above half saturation, Michaelis-Menten behavior is observed (Fig. 1B). This unusual behavior can be explained by the symmetry model of allosteric enzymes (10) if an interaction of a high number of identical subunits

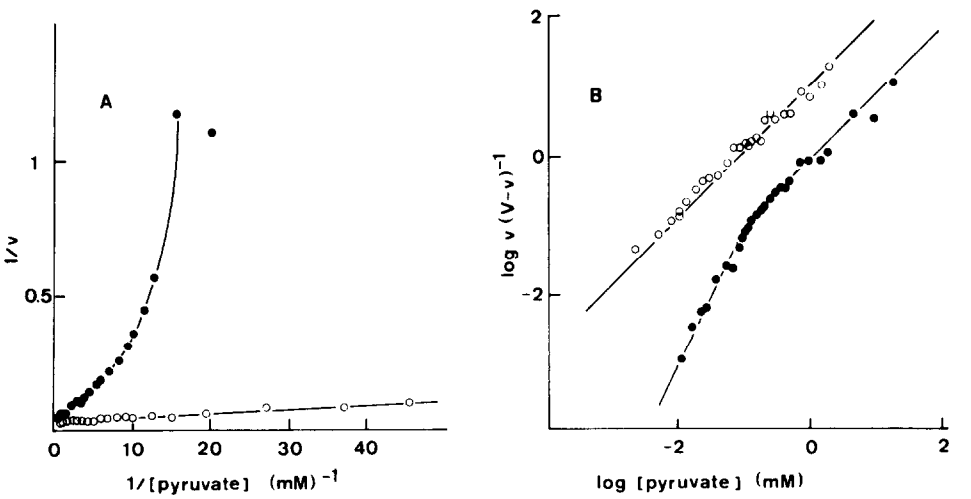

Fig. 1: Dependence of the initial reaction rate of the bacterial and the mammalian pyruvate dehydrogenase complexes on pyruvate concentration. The enzyme activity was tested with 0.26 µg per ml of enzyme form E. coli (●) and with 2.2 µg per ml of enzyme complex from bovine kidney (○). A, Lineweaver-Burk diagram; B, Hill diagram.

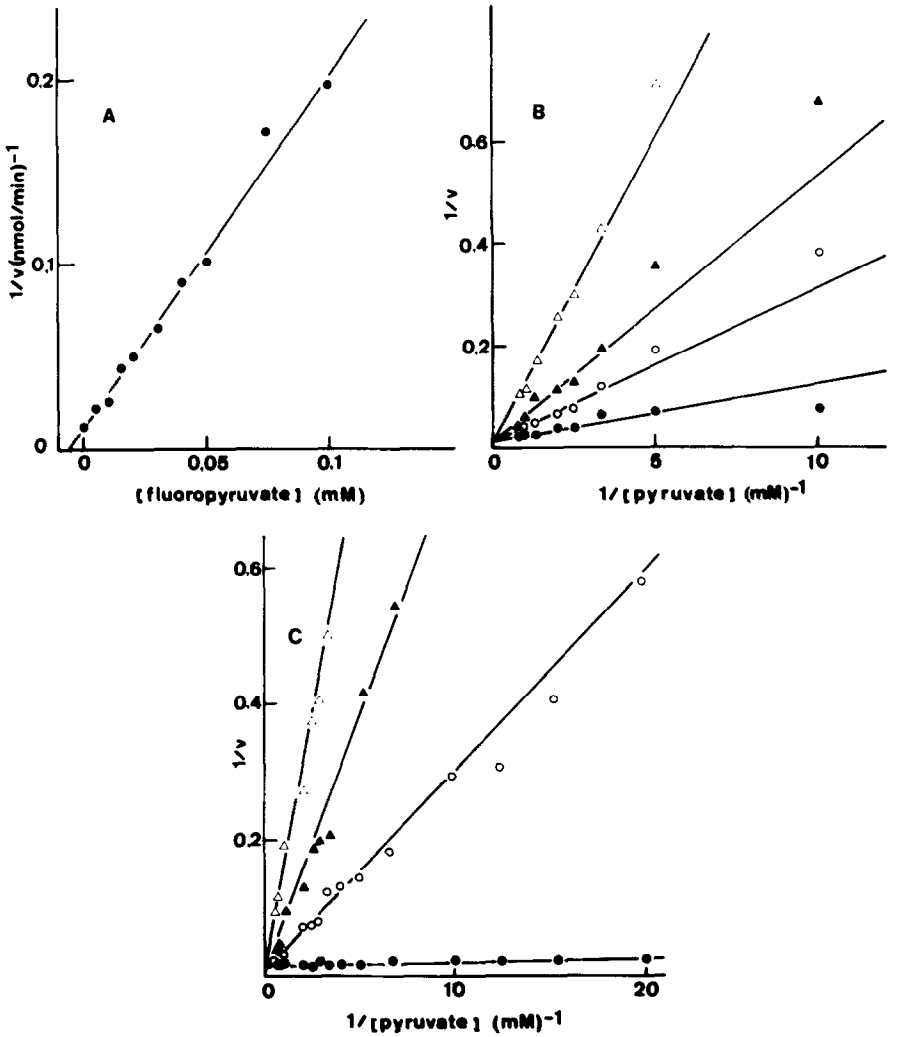

Fig. 2: Inhibition of initial reaction rates of the pyruvate dehydrogenase complex by fluoropyruvate. A, increasing amounts of fluoropyruvate concentration at a constant amount (2 mM) of pyruvate, measured with 2.4 µg of the enzyme complex from E. coli. B, increasing amounts of pyruvate concentration in the absence (●) and in the presence of 2 (○), 5 (▲) and 12.5 (△) µM fluoropyruvate, measured with 1.6 µg per ml of the enzyme complex from E. coli. C, the same experiment as in B with 3.3 µg per ml of the enzyme from bovine kidney. Fluoropyruvate concentrations: 0 (●), 0.1 (○), 0.2 (▲) and 0.5 mM (△) respectively.

is considered (11). In contrast, no cooperative effect can be seen with the enzyme complex from bovine kidney (Fig. 1). The K_m - value of the mammalian enzyme (0.06 mM) is considerably lower than that of the enzyme complex from bacteria (K_m = 0.3 mM).

Inhibition by fluoropyruvate. The overall enzymatic activity of the pyruvate dehydrogenase complexes from both bacterial and mammalian sources is strongly inhibited by fluoropyruvate. The inhibition is competitive to pyruvate (Fig. 2). To demonstrate the inhibitory effect in the case of the bacterial enzyme complex, the Michaelian range of the saturation curve was regarded. From these values the inhibition constant was calculated to be 1.4 μM, while the respective value for the mammalian enzyme was somewhat higher (K_i = 3.6 μM).

In order to understand more of the cooperative mechanism of the pyruvate dehydrogenase complex from E. coli, it was of interest to see whether the substrate analog bound to the enzyme in a similar way as did the pyruvate. In Fig. 2A, however, normal binding behavior is clearly shown for fluoropyruvate when the enzymatic activity is tested in the presence of increasing amounts of the inhibitor.

Reversibility of the fluoropyruvate inhibition. Since fluoropyruvate is able to covalently bind to sulfhydryl groups (12), it is necessary to distinguish whether the observed inhibitory effect is due to a competition for the substrate binding site or whether this inhibition is caused by irreversibly blocking one of the catalytic centers of the pyruvate dehydrogenase complex. To explore this problem further pyruvate dehydrogenase complexes from both of the above sources were incubated with fluoropyruvate at a concentration which brings about very nearly complete inhibition of the enzymatic activity. The incubation was done in the absence as well as in the presence of pyruvate, thiamine diphosphate and magnesium ions. These factors cause a reduction of the transacetylase-bound lipoic acid which in turn becomes accessible for SH-reagents (13, 14). Control experiments were carried out by omitting fluoropyruvate from the incubation mixture. At different times, aliquots were taken and tested for the level of

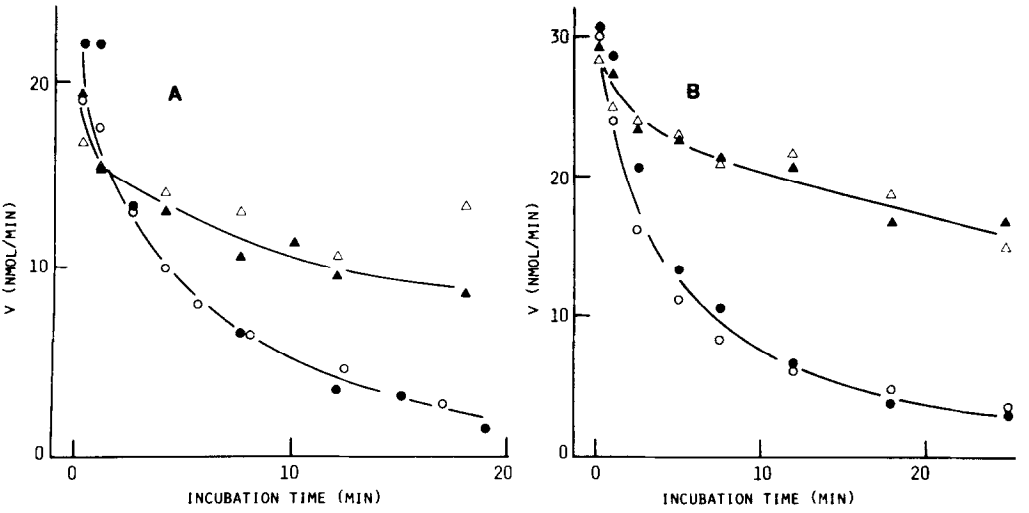

Fig. 3: Test of the reversibility of fluoropyruvate inhibition. Pyruvate dehydrogenase complexes from E. coli (A, 0.16 mg per ml) and from bovine kidney (B, 0.22 mg per ml) were incubated at 37° C in the presence of 0.1 M potassium phosphate buffer pH 7.6 under the following conditions: a) enzyme complex alone (▲); b) enzyme complex plus fluoropyruvate (10 µM in A and 50 µM in B) (△); c) enzyme complex plus 0.3 mM pyruvate, 0.2 mM thiamine diphosphate and 1 mM magnesium chloride (●); d) enzyme complex plus fluoropyruvate (10 µM in A and 50 µM in B), 0.3 mM pyruvate, 0.2 mM thiamine diphosphate and 1 mM magnesium chloride (○). After the times indicated 10 µl aliquots were diluted in 1 ml of the test mixture and the enzyme activity was measured immediately. The pyruvate concentration in the test was 0.3 mM.

enzyme activity. As can be seen (Fig. 3) no difference in the enzymatic activity can be observed whether fluoropyruvate is present in the incubation vessel or not. This is true for the enzyme complexes from both sources. A remarkable inactivation occurs, however, during the preincubation period especially if pyruvate and thiamine diphosphate are present.

DISCUSSION

The regulation of the catalytic activities of the mammalian and the bacterial pyruvate dehydrogenase complexes appears to occur by different regulatory mechanisms. The predominant regulatory mechanism of the mammalian enzyme complex seems to be the reversible phosphorylation-dephosphorylation process (15) while no such control principle was

found for E. coli. The complicated phosphorylation-dephosphorylation system could be viewed as an evolutionary achievement of the mammalian complex while the allosteric control observed in the bacterial enzyme could represent a more primitive regulatory mechanism.

Fluoropyruvate obviously is a very potent inhibitor of the bacterial and of the mammalian pyruvate dehydrogenase complex and a competitive mechanism is seen in both cases. The apparent affinity for the inhibitor is 20 - 200 times that of the substrate. This indicates a strong binding to the enzyme, a fact that cannot easily be understood. Since fluoropyruvate should exist largely in the bulky diol-form (16), the strong binding of the inhibitor seems to contradict the demand for high specificity that has to be invoked for the substrate binding site. A possible explanation for the increased affinity of the inhibitor, however, may come from the fact that interaction occurs between the fluorine atom and those electrophilic groups of the enzyme that are responsible for the binding of the carbonyl and the carboxyl groups (7).

The very strong inhibition of the pyruvate dehydrogenase complex by fluoropyruvate makes this enzyme the main target for the toxic effect of the inhibitor in cell metabolism. Mager and Blank (17) found fluoropyruvate to be one tenth as toxic as fluoroacetate in mice, but to have a strong inhibitory effect on the growth of bacteria. This reduced toxicity may be explained by the different metabolic effect of both inhibitors. Fluoroacetate itself does not inhibit but is metabolized in the lethal product fluorocitrate which inhibits the citrate condensing enzyme (4, 5).

The partial inactivation of the pyruvate dehydrogenase complex upon preincubation at 37° C may be caused by thermal denaturation of the

enzyme in highly diluted solutions. One interesting aspect is the observation that neither the substrate nor the cofactors are able to prevent the enzyme from denaturation, rather they accelerate this process. It seems possible therefore that the reduction of the enzyme-bound lipoic acid causes the complex to be more susceptible to denaturating influences.

ACKNOWLEDGMENTS

I wish to thank Dr. Patric A. Clapshaw and Mrs. Ursula Bantel-Schaal for the help in the preparation of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

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